PHYCOMYCETES RECOVERED FROM SOIL SAMPLES COLLECTED BY W. R. TAYLOR ON THE ALLAN HANCOCK 1939 EXPEDITION*

(WITH TWO PLATES)

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Through the kindness of Professor W. R. Taylor soil samples were obtained from certain Central and South American localities visited by him during the 1939 Allan Hancock Expedition. Water cultures were prepared from each of these samples and "baited" with appropriate substrata. Although the extreme dryness of the soil samples when received undoubtedly restricted greatly the variety and number of fungi still in a viable condition, they nonetheless yielded species of exceptional interest. Indeed, one of these, *Monoblepharella Taylori,* was found to be unique among plants in the behavior of the egg after fertilization.

All the fungi recovered are known to possess a resting spore stage resistant to unfavorable environmental conditions, such as drought, which would be fatal to the vegetative parts. It is easy, therefore, to understand how they survived the vicissitudes to which they were necessarily exposed from the time of their collection until they reached—some months later—the more congenial habitat of a battery jar of sterile water provided with bits of substrata.

The samples were collected into clean new boxes by careful methods, using ordinary precautions against the possibility of mixture. They were not, however, collected or stored under strictly aseptic conditions. The collection boxes were taped shut immediately after collection and remained unopened until they reached the laboratory. Pieces of soil were then cut out with a sterile scalpel from the middle of each sample and placed in jars of sterile water containing bits of "bait."

The following fungi were recovered:

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Chytridiales

1. *Rhizophidium carpophilum* (Zopf) Fischer

Parasitic on oospores of *Achlya sp.*[1] Sandy bank of a stream ("Site I"). Caledonia Bay, Panama; April 26, 1939.

A species hitherto known only from the Eastern United States, Western Europe, and Japan.

Blastocladiales

2. *Allomyces javanicus* Kniep

Mud from a roadside watering trough on the road from Curacas to La Guaira, Venezuela; April 11, 1939.

According to F. T. Wolf (1939), no epigynous species of *Allomyces* have been reported from the Western Hemisphere. In connection with the present record, it might be mentioned that this species has also been isolated from soil from Texas, where it was found associated with *Blastocladiella simplex* Matthews.

The gametophyte differs from that formed by subcultures of Kniep's Javanese material in the occasional formation in water cultures of extraordinarily long, cylindrical female gametangia.

3. *Allomyces moniliformis* Coker and Braxton

Soil from a paddy field on the road to Pitch Lake from Port of Spain, Trinidad, B.W.I.; April 18, 1939.

This rare species has hitherto been known only from the Eastern United States (two collections) and Mexico (Wolf, 1939).

4. *Allomyces* sp. indet.

Soil from a spring on a hillside above the village of Bahia Honda, Panama; March 28, 1939.

So far, this isolate, which resembles in its sporophyte phase *A. javanicus* and *A. arbuscula,* has failed to produce a gametophyte. It is possibly *A. anomala* Emerson, inedit., an unpublished species which, according to Wolf (1939), is to include short-cycled species lacking both a gametophyte and "Cystogenes" phase.

[1] Attempts are being made to obtain normal material of this fungus for purposes of identification.

Monoblepharidales

5. *Monoblepharella* n. gen.

Mycelium, contents, zoosporangia, zoospores, oogonia, antheridia, and antherozoids as in *Monoblepharis,* the egg after fertilization emerging from the oogonium and by means of the persistent flagellum of the male gamete undergoing a period of swarming, after which it encysts and becomes a thick-walled oospore; the oospore upon germination forming the vegetative mycelium.

Mycelium, sporangia, zoosporis, oogonium, antheridia, antherozooideis simuli *Monoblepharis;* ova inseminata postice uniciliata, natantia; oosporis in aqua liberis, germinatio formati mycelio.

Monoblepharella Taylori comb. nov.

Monoblepharis Taylori Sparrow,[2] *in* Mycologia 31: 737. 1939.

Mycelium well developed, consisting of tenuous, flexuous branched hyphae 2—5μ in diameter, the contents reticulately vacuolated; sporangia narrowly siliquiform with a tenuous wall, variable in size, 35—65μ long by 5—9μ in diameter, with a very narrow (2.5—4μ) base, occurring singly or in pairs at the tips of the hyphae or after sympodial branching of the hypha appearing lateral; zoospores ovoid or somewhat cylindrical, 7—9μ long by 4.5—5μ wide, the posterior cilium 2—3 times the length of the body; oogonium at first terminal or after sympodial branching of the supporting hypha often appearing lateral, clavate or obpyriform with rounded apex and narrow cylindrical base, 15—17μ long by 8—10μ wide, tapering to 2—3μ at the base, the contents at maturity forming one or occasionally up to 6 eggs containing numerous large refractive globules; antheridium usually hypogenous, several often developed in basipetal succession, consisting of a cylindrical segment of the suboogonial hypha and a beaklike lateral outgrowth 8—10μ long by 4—5μ wide, antherozoids two to five, strongly amoeboid, posteriorly uniciliate, ovoid when swimming and about 5μ long by 3μ wide, escaping through a pore formed at the tip of the beak; zygote broadly ovoid to nearly spherical, 10—13μ long by 8—10μ wide, posteriorly uniciliate, free swimming, the contents bearing numerous large refractive globules; oospore formed free in the water, spherical, 8—11μ in diameter, with a slightly thickened, light brown, smooth wall, contents bearing globules, upon germination forming a mycelium.

[2] A Latin, but not an English, description has been previously published in Mycologia.

In soil, from a paddy field on the road from Port of Spain to Pitch Lake, Trinidad, B.W.I., April 18, 1939; from sandy soil on the bank of a stream ("Site I"), Caledonia Bay, Panama, April 26, 1939; dry bed below waterfall, 9 miles from Madden Dam, Panama, Canal Zone, March 31, 1939.

After a careful consideration and study of the genus *Monoblepharis* it has seemed advisable to segregate *M. Taylori* in a genus of its own. The remarkable behavior of the zygote is unlike anything observed in *Monoblepharis,* or, indeed, in the Fungi or Algae, and well warrants generic distinction. It is possible that *M. ovigera* and *M. regignens* may in the future be found to possess this same type of sexual reproduction, in which case they too might be included in *Monoblepharella.* While *Monoblephariopsis* already exists for their accommodation, this genus is founded on fungi known only from the imperfect stage. Since *Monoblepharella* is based on a species with both perfect and imperfect stages, it would, rightly, take precedence.

The thallus and sporangia of *M. Taylori* resemble in superficial aspect those of *Monoblepharis regignens* and *M. ovigera.* The mycelium, which forms a lustrous, pearly gray halo around the substrate, is composed of delicate, moderately branched hyphae 2—3μ in diameter. Near the base, where the plant is anchored by a system of holdfasts to the substrate, the hyphal axes may attain a diameter of 5μ. Catenulate series of swellings are formed on the hyphae of some, but not all, isolates. It is suspected that these are due to an extraneous parasitic organism. However, no reproductive phase has ever been observed which would substantiate this idea. The contents of the hyphae are characteristically disposed in a rich network, or reticulum, within which may be seen moving along the long axis somewhat coarse refractive granules of irregular size. A preliminary cytological examination of these hyphae shows the minute nuclei to be disposed at more or less regular intervals.

Occasionally both nonsexual and sexual reproductive organs may be formed simultaneously on the same plant (pl. 17, fig. 10). However, at room temperature (20-21°C.) a preponderance of zoosporangia is produced, whereas at 30°C. the formation of sexual organs occurs in abundance.

The zoosporangia are ordinarily produced at the periphery of the colony at the tips of delicate, sparingly branched hyphae. By subsequent sympodial branching of the hypha they come to appear lateral. The difference in width of the sporangium and its attendant hypha is so striking that the former frequently appears as though it were a long, slender fusiform or siliquiform highly refractive conidium lying free in the tangled mycelial complex. The sporangia vary from 35—65μ long by 5—9μ wide, the base generally tapering to 2.5—4μ. The zoospores are fully matured before discharge and emerge (pl. 17, fig. 9) through a small pore formed upon the deliquescence of the sporangial apex, in the same manner as in species of *Monoblepharis* (Sparrow, 1933). They are ovoid or somewhat cylindrical, 7—9μ long by 4.5—5μ wide, and possess a single long posterior cilium. The internal organization is exactly like that found in *Monoblepharis*.

Oogonia and antheridia are frequently formed on somewhat shorter branches of the thallus than are the zoosporangia. The clavate or obpyriform oogonium, 15—17μ long by 8—10μ wide, with a narrow base 2—3μ in diameter, may, like the sporangium, be at first terminal but, after sympodial branching of the hypha, appears lateral. Both oogonium and antheridium develop in the same manner as do those of *Monoblepharis sphaerica* (Sparrow, 1933), i.e., the rudiment of the terminal oogonium is formed first. After this is delimited, another, more proximal segment is separated from the supporting hypha by a cross wall. In most cases this basal segment before its delimitation has formed a short branch beneath the oogonium which continues to increase in size as maturation proceeds. The mature oogonium is thin walled and apparently without a prominent receptive papilla, although further observations are needed on this point. The contents of the large, broadly ellipsoidal egg are made highly characteristic and conspicuous by the possession of numerous large, colorless, refractive globules (pl. 16, fig. 1), embedded in the clear cytoplasm. Although in most cases only a single egg is formed in the oogonium, 2—6 have occasionally been found (pl. 17, fig. 8). The mature antheridium, which may be formed singly or in basipetal series beneath the oogonium, consists of a cylindrical portion and a large, lateral, beaklike outgrowth, 8—10μ long by 4—5μ wide, formed from the previously mentioned branch. About 2—5 strongly amoeboid, posteriorly uniciliated antherozoids, 5μ long by 3μ wide, are produced which escape through a pore formed at the tip of the beak. These may creep about after discharge or, like the zoospores which they resemble in all but size, swim about in the medium. The early stages in the process of fertilization are like those found in *Monoblepharis*. The antherozoid after reaching the apex of the fully mature oogonium becomes strongly amoeboid. Its contents become watery and spread over the oogonial apex (pl. 16, figs. 1-4). Numerous small vacuoles appear and disappear so rapidly as to give an appearance of cytoplasmic "boiling." The cilium waves feebly above the body of the sperm and, as the cytoplasm of the male gamete gradually sinks into the ooplasm, becomes more hyaline in appearance. During absorption of the male gamete the ooplasm expands and for a short time fills the oogonium (pl. 16, figs. 5, 6). In none of the many cases of fertilization observed was the body of the male gamete completely engulfed by the ooplasm. There always remained a small, papillalike part at the apex, from which protruded the cilium of the antherozoid (pl. 16, fig. 6). The remainder of the male gamete could be detected for a time as a more slightly granular material in the anterior part of the egg, but it was soon lost to view. Almost instantly after the absorption of the major part of the antherozoid, the papillalike residue of this structure on the surface of the egg started to increase in size (pl. 16, fig. 7). This marked the initiation of evacuation of the zygote from the oogonium. More definite evidence of emergence could then be seen in the migration of the large, conspicuous globules into the enlarging papilla (pl. 16, figs. 8-10). These continued to flow out with the cytoplasm of the zygote. Meanwhile, the cilium remained passive and, as the zygote continued to ooze out, slowly assumed a lateral position with respect to the orifice of the oogonium. The completely emerged zygote was at first somewhat pyriform, with the cilium nearly basal and extending at a right angle or more to the long axis of the body (pl. 16, fig. 11). It then rounded off (pl. 16, fig. 12), remained quiescent for a few seconds, and then began to rock gently. A trembling movement of increasing intensity was soon initiated, which frequently carried it away somewhat from the oogonial orifice (pl. 17, fig. 1). Lateral vibration of the hitherto quiescent cilium then occurred, and vacuoles appeared in the anterior part of the now more ovoid body (pl. 17, fig. 2). After a few violent tugs, accompanied by rapid vibration of the dark-appearing cilium, rotation of the zygote on its long axis as well as forward progression was initiated, and it slowly swam off. Under poor environmental conditions the zygote may fail to emerge, and the oospore is formed in the oogonium (pl. 17, fig. 7).

After a period of motility of unknown duration, frequently punctuated by periods of quiescence and strong amoeboid crawling (pl. 17, fig. 3), the zygote comes to rest. Its cilium is apparently absorbed (pl. 17, fig. 4), and the body becomes surrounded by a thickened wall (pl. 17, fig. 5). The globules persist for a time, but eventually these are absorbed and the oospore undergoes a period of rest, far removed from the oogonium. The precise duration of encystment is not known. Fully mature oospores dried for three weeks on cover slips have germinated when placed in water. It will also be recalled that the soil samples from which the fungus was isolated had been dry at least two months before they were obtained for study. It is probable, therefore, that the oospore can remain viable in the soil during ordinary periods of tropical drought.

Upon germination, a single small pore is formed in the oospore wall, through which a hypha emerges (pl. 17, fig. 6). The latter elongates indefinitely, branches, makes contact with bits of organic material, and re-establishes the fungus. Reproductive organs of either type may be formed, or the mycelium may continue its vegetative growth.

Monoblepharella Taylori presents among other features a type of sexual reproduction of unusual interest. So far as now known, sexuality in those uniciliated Phycomycetes where one or both the gametes are free swimming may be isogamous *(Olpidium, Synchytrium, Blastocladiella variablis),* anisogamous *(Allomyces javanicus, A. arbuscula),* or oogamous *(Monoblepharis).* In this series one type of sexual reproduction has not as yet been found, namely, that in which a free-swimming egg is fertilized out in the water by a motile sperm. When *M. Taylori* was first discovered, it was thought that it might possess this sort of reproduction. A close examination, however, revealed otherwise, for it is not the egg that is ciliated and motile, but the *zygote.* It seems highly probable, therefore, that there exists in nature an oogamous organism of this group in which both gametes are motile.

6. Phycomycete of unknown affinities.

Sandy bank of a stream ("Site I"), Caledonia Bay, Panama; April 26, 1939.

Only a few thalli and mature sporangia of this fungus were found. All attempts to multiply it failed, and hence little is known of its life history or affinities.

The plant consists at maturity of two well-defined parts: a distal, more or less spherical body, 70—200μ in diameter with a broad discharge tube 15—30 x 50—60μ long, and, continuous with it, a trunklike basal stalk, 50—220μ long x 25μ wide, from the tip of which emerges a series of sparsely or richly branched holdfasts. Occasionally the stalk is lacking and the holdfasts arise directly from the body. The two parts are separated by a cross wall. The contents of the swollen apex and tube become segmented—apparently by successive division—into an extremely large number of roundish or somewhat angular spores about 10μ in diameter. The cytoplasm of these bodies consists of a granulated matrix, within which are embedded a few bright granules. No spore discharge was ever witnessed. The frequent finding of columns of motionless spores in the medium seemed to indicate that they were set free upon the disintegration of the wall of the discharge tube.

In its superficial aspect the fungus resembles a species of *Blastocladiella*. An even closer resemblance to the recently described *Rhizidiomyces bivellatus* (Nabel, 1939) is found not only in the presence of a discharge tube but in the occasional lack of a stalk. Nabel's fungus was discovered in soils collected in Haiti, Venezuela, Mexico, and Yugoslavia.

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EXPLANATION OF PLATES

All figures were drawn from living material with the aid of the camera lucida. The absolute scale is given on each plate.

PLATE 16

Stages in the fertilization of the egg of *Monoblepharella Taylori* and the emergence of the zygote.

FIGS. 1, 2. Emergence of the last of four antherozoids from the antheridium.

FIGS. 3-6. Absorption of the antherozoid by the egg. In Fig. 6 all but a small portion of the male gamete and its cilium has sunk into the ooplasm. The cilium has assumed a lateral position.

FIG. 7. Beginning of emergence of the zygote.

FIGS. 8-10. Further stages in emergence.

FIG. 11. Completely emerged, somewhat ellipsoidal zygote resting at the mouth of the oogonium.

FIG. 12. Zygote assumes a more spherical shape and moves away from the orifice of the oogonium.

ERRATA: Plates 16 and 17 are transposed. With this correction, all references to both plates are accurate.

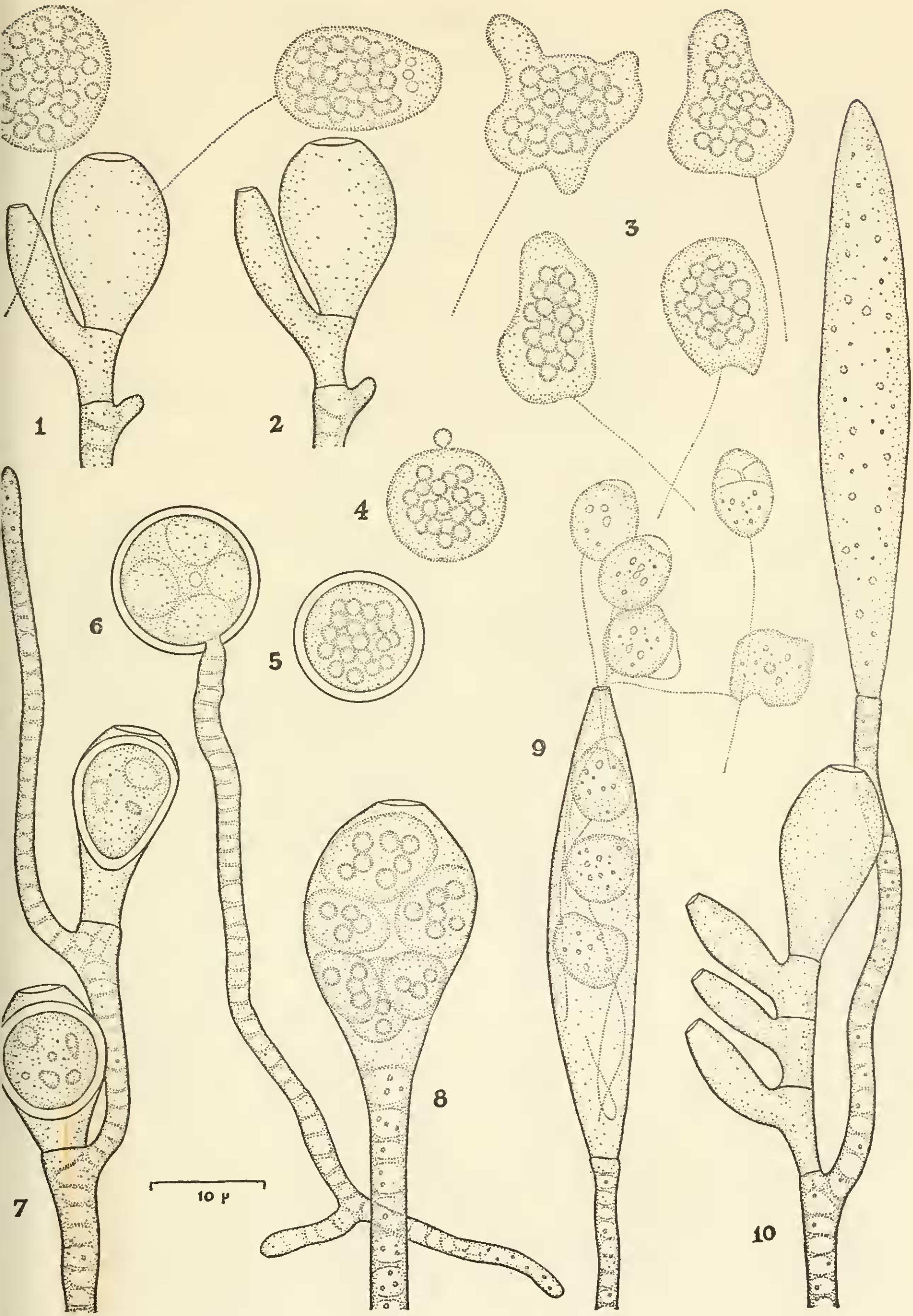
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PLATE 17

Figs. 1, 2. Final stages in the escape of the zygote. In Fig. 2 the body has started to rotate on its long axis, and the cilium is in motion.

Fig. 3. Amoeboid changes of shape undergone by the zygote during temporary periods of rest.

Fig. 4. Zygote at rest. The body has become rounded and the cilium condensed into a small droplet.

Fig. 5. Mature oospore free in the water.

Fig. 6. Germinated oospore. The full length of the hypha is not shown.

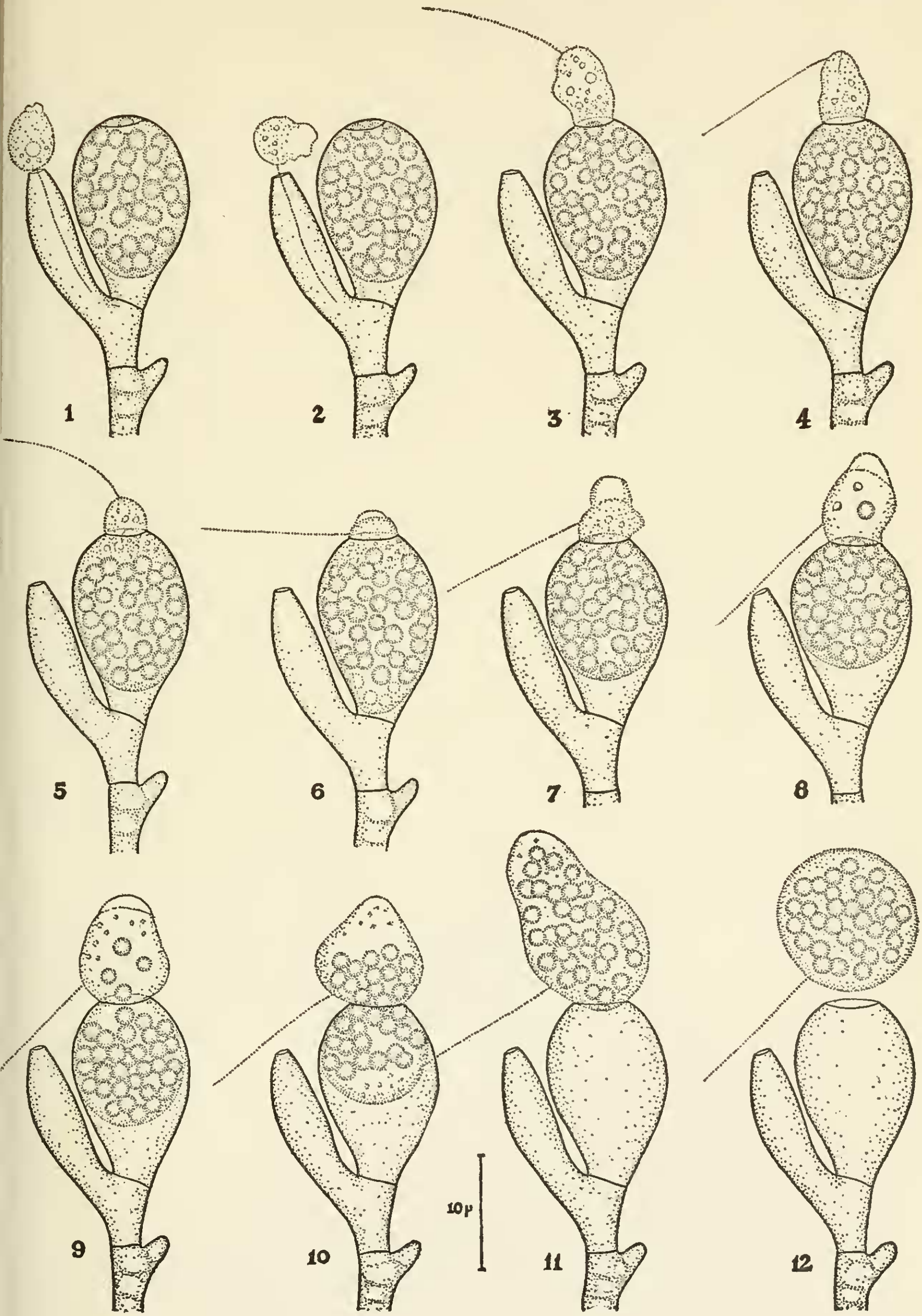
Fig. 7. Two oospores formed within the oogonium. Occurring commonly under poor environmental conditions.

Fig. 8. Unusually large oogonium containing six eggs.

Fig. 9. Discharging zoosporangium. A mature zoospore with the typical internal structure assumed during motility is shown in the upper right figure.

Fig. 10. Portion of a hyphal tip bearing an immature zoosporangium and an empty oogonium beneath which are several antheridia.

Figures inked in by Richard Higgins.



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